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NOTES ON THE FECUNDATION OF ZAMIA AND THE  
POLLEN TUBE APPARATUS OF GINKGO.

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(WITH PLATE X)

A PRELIMINARY discussion of the development of the pollen tube apparatus<sup>1</sup> and the antherozoids<sup>2</sup> of *Zamia* was given by the writer in the June and July numbers of this journal. The object of the present preliminary paper is to call attention to some of the peculiar phenomena which occur during the process of fecundation in *Zamia*, and to certain features in the development of the pollen tube apparatus of *Ginkgo*, in which further light is thrown on the origin of the centrosome-like body occurring here as in *Zamia*.

For a considerable time during the development of the pollen tube apparatus in *Zamia* the archegonium remains in nearly the same stage of development, simply increasing gradually in size. During this period the very large nucleus remains at the apex of the archegonium as figured by Treub in *Cycas circinalis*.<sup>3</sup> Shortly before fecundation this nucleus divides, and

<sup>1</sup> Peculiar structures occurring in the pollen tube of *Zamia*. BOT. GAZ. 23:453. June 1897.

<sup>2</sup> The development of the antherozoids of *Zamia*. BOT. GAZ. 24:16. July 1897.

<sup>3</sup> Recherches sur les Cycadées. Ann. du Jardin Bot. de Buitenzorg 4: pl. 1. fig. 8.

a small cell is cut off at the apex of the archegonium, which corresponds to the canal cell of the conifers. Until the publication of Ikeno's preliminary note announcing the discovery of this canal cell in *Cycas revoluta*<sup>4</sup> it had been supposed that it was not formed in the Cycadaceæ. It would seem, however, from its occurrence in *Cycas* and *Zamia* that it is commonly formed in the Cycadaceæ as in the Coniferæ. Hirase has also recently described the formation of this cell in *Ginkgo biloba*. I have not observed the division of the nucleus leading to the formation of the canal cell in *Zamia*, but the process probably corresponds very closely to that occurring in *Cycas* and *Ginkgo*. Before fecundation the canal cell appears to break up and lose its identity, as only occasional traces of it can be found at that time.

After the division which leads to the formation of the canal cell, the lower nucleus, which forms the oosphere, travels downward and takes a position somewhat below the middle of the archegonium. It is usually spherical or slightly elliptical, and its contents are much less dense than the surrounding cytoplasm of the archegonium with which it forms a marked contrast. The mature archegonium is usually elliptical or slightly reniform and is about 3<sup>mm</sup> in length and 1 to 1.5<sup>mm</sup> in width. As explained in my previous papers, several antherozoids commonly enter each archegonium, two being usually found and sometimes three or four. The entire antherozoid enters unchanged, swimming in between the ruptured neck cells. Only one of the antherozoids is concerned in fecundation, and the others are usually found between the protoplasm and wall of the archegonium, presenting their original form and appearance, or in some stage of disintegration. Occasionally one of the antherozoids not concerned in fecundation pushes for a short distance into the contents of the archegonium, but it apparently does not mingle with the protoplasm of the archegonium, as it is always found in such cases to form a distinct body which stains very differ-

<sup>4</sup>Note préliminaire sur la formation de la cellule de canal chez le *Cycas revoluta*. The Botanical Magazine 10:61. September 1896.

ently. The antherozoid which is utilized in fecundation swims into the protoplasm of the archegonium for a short distance, where it undergoes a remarkable change. In very numerous sections shortly after fecundation the spiral ciliiferous band of the antherozoid which, it will be remembered, is developed by the gradual extension of the membrane of the centrosome-like body, appears uniformly lying in the protoplasm at the apex of the archegonium. It shows very plainly and presents nearly the original form of the antherozoid (*fig. 3*), but all traces of the nucleus and cytoplasm, which originally made up the main body of the antherozoid, have disappeared. The band, preserving its original spiral form, now lies free in the protoplasm of the archegonium. No instance has been found of the occurrence of more than one antherozoid presenting this appearance, and in every fecundated archegonium carefully examined one of these bands has been found. Since it was evident from this that the nucleus of this antherozoid must be the one utilized in fecundation, search was made for intermediate stages. Fortunately several have been found which support this view of the matter. In three different cases, immediately in the rear of the isolated spiral ciliiferous band described above, a nucleus has been found which, judging from its size and appearance, is evidently the nucleus of this antherozoid (*figs. 1 and 2*). I have been unable to determine the fate of the cytoplasm which surrounded the nucleus in the original antherozoid form, but from the slightly different density and constitution of the protoplasm which now lies between the spirals of the ciliferous band, it would seem that it simply unites with that of the archegonium. I have thus far been unable to find any other intermediate stages in the passage of the male nucleus, but it may be assumed from the above observations that shortly after the antherozoid enters the protoplasm of the archegonium, the nucleus escapes from the body of the antherozoid and from this point wanders alone to the oosphere. After fecundation the male nucleus appears as a small nearly round body in the upper portion of the oosphere into which it has penetrated (*fig. 3*).

The contents of the male nucleus is at this time much more dense than that of the oosphere.

The isolated ciliiferous band lying free in the protoplasm at the apex of the archegonium evidently retains its identity for a considerable time. It has been observed in several archegonia after the formation of many free nuclei by the repeated divisions of the oosphere. Frequently the spindles of some of these free nuclei in division have been observed between its spirals. The band ultimately disappears, its substance probably being consumed by the forming embryo. The primary function of the ciliiferous band thus certainly ends with the transporting of the male nucleus from the pollen tube to the archegonium. The exceptional size of the antherozoids of *Zamia* permits these features to be seen very plainly, while in the various plants in which the entrance of the antherozoids has been studied, they are so small that thus far the fate of the cilia and cytoplasm, which are not generally supposed to be concerned in fecundation, has not been determined with certainty. Professor Strasburger,<sup>5</sup> in his recent study of the fecundation of *Fucus*, concludes, mostly from comparative size, that shortly after the entrance of the antherozoid its cytoplasm unites with that of the egg cell, and only the nucleus continues its passage and unites with the egg nucleus. My observations clearly indicate that this is the case also in *Zamia*.

A special examination has been made of the divisions of the oosphere immediately after fecundation for the occurrence of a centrosome which, if present, would be suggestive in connection with the centrosome-like body of the antherozoid. Thus far, however, I have been unable to find any indication of such an organ. Careful examinations have been made of dividing nuclei in many stages of development from the second division of the oosphere until the embryo is fairly well formed. The first division of the oosphere has not been observed. In many cases the kinoplasm may be found presenting somewhat the appearance

<sup>5</sup> Kerntheilung und Befruchtung bei *Fucus*. Jahrbücher f. wissenschaft. Bot. 30: 363. 1897.

of an aster, but in no case has any body been noticed which could be considered a centrosome. Hirase, in his studies of Ginkgo, states that he was unable to find any indication of a centrosome either in the first or the later divisions of the oosphere.<sup>6</sup> It should be remembered in this connection that Overton<sup>7</sup> states that in *Ceratozamia* the pollen mother cells and endosperm shortly after the formation of the free cells in the embryo sac are very favorable objects for the study of the centrosome. It would seem, however, from Hirase's studies on Ginkgo, my own on *Zamia*, and the recent cytological studies at the Bonn Botanical Institute, that Overton may possibly have been mistaken.

In the generative cell of *Ginkgo biloba*, according to Hirase,<sup>8</sup> two "attractive spheres" occur which are visible without staining. These organs are unquestionably the same as the centrosome-like bodies which I have described in *Zamia*. Hoping thus to obtain further information as to the nature and origin of these bodies, I have made a study of the early stages of the development of the pollen tube of Ginkgo. In developing fruits collected June 12, the pollen was found to have germinated and formed a tube about as long as the diameter of the pollen grain. In this stage (*fig. 4*) the vegetative nucleus in all cases examined had already wandered into the tube and was commonly found near its distal end. The stalk cell and generative cell, which, as Strasburger has shown, are formed in the pollen grain before pollination, had at this period increased only slightly in size, and were yet much smaller than the vegetative nucleus. The generative cell in this stage is crescent shaped and projects into the center of the pollen grain. The nucleus is about  $5.6\mu$  in diameter and occupies about one-half of the cell. The nucleus of the stalk cell is somewhat smaller, while the vegetative nucleus is much larger, being about  $10.5\mu$  in diameter.

<sup>6</sup> Études sur la fécondation et l'embryogénie du *Ginkgo biloba*. Journ. of the Col. of Sci. Imp. Univ. Japan 8 : 307.

<sup>7</sup> Ueber die Reduction der Chromosomen in den Kernen der Pflanzen. Vierteljahrschr. d. naturforsch. Ges. in Zürich 38 : 178.

<sup>8</sup> Notes on attraction spheres in the pollen cells of *Ginkgo biloba*. The Botanical Magazine 8 : 359.

During June and the early part of July the pollen tube continues gradually to grow in diameter, increasing but slightly in length, until by the middle of July it has reached a diameter somewhat greater than the pollen grain, which at this time forms simply a cap over the proximal end of the tube (*fig. 5*). The generative and stalk cells have increased greatly in size and have pushed out of the pollen grain into the tube, but still remain attached. A careful search was made in the generative cells of material collected at various periods up to this time for the centrosome-like bodies or "attractive spheres" described by Hirase, but in no case was any indication of them discovered. In material collected July 20, however, the centrosome-like bodies were found for the first time (*figs. 5 and 6*). There is considerable variation in the rapidity of development of different pollen tubes in fruits collected the same day, and it was only in the most advanced tubes that the centrosome-like bodies could be found at this time. In material collected July 27 they were found in almost every generative cell examined. Two of the spheres occur uniformly in each generative cell and are located in the cytoplasm on opposite sides of the nucleus about midway between the nuclear membrane and the cell wall (*fig. 6*). They are spherical and of uniform size, but are quite small, being only about 0.6 to 0.7 $\mu$  in diameter. They are very plainly distinguishable, however, as they stain differently from the surrounding cytoplasm. The radiations of kinoplasm are at this time few in number and comparatively short. They are thick at the base where they join the so-called centrosome-like bodies and are reduced to a very fine point at the end. The generative cell in this stage is usually nearly spherical, being somewhat compressed on the side attached to the stalk cell. It varies in size, being commonly from 25 to 35 $\mu$  in diameter. The nucleus is spherical, from 16 to 18 $\mu$  in diameter, and contains a nucleolus. Both the nucleolus and the centrosome-like body are stained bright red with saffranin in using the Flemming triple stain, as in the case of the centrosome-like body and nucleolus of *Zamia*.

During August the stalk cell and generative cell continue

to increase gradually in size. In material collected August 30 the generative cell was found to have become elliptical in shape, its dimensions being now about 45 by 65 $\mu$  (*fig. 7*). The nucleus, on the contrary, had become compressed and was now fusiform instead of spherical, its major axis being at right angles to the major axis of the cell. It was commonly about 45 $\mu$  in length and 18 $\mu$  in width. The centrosome-like bodies had increased in size, being now from 2 to 2.5 $\mu$  in diameter. The radiating filaments of kinoplasm were still few and short as in the preceding stage. Between the centrosome-like body and the nuclear membrane on each side of the nucleus a regular spherical body of nearly uniform size, 7 to 8 $\mu$  in diameter, had made its appearance. It stained bright red with saffranin, the same as the nucleolus and centrosome-like body, but not quite so intensely. The nature and function of these nucleolus-like bodies are still in doubt. That they are not caused by the action of fixatives is quite clear, as Hirase<sup>9</sup> observed them in living unstained generative cells. Furthermore, I find them uniformly in generative cells at this stage, while in a slightly younger stage they do not occur. Zimmermann<sup>10</sup> being unable to account for these bodies suggested that what Hirase supposed to be the nucleus was in reality the generative cell, and that these two nucleolus-like bodies were to be considered as vegetative nuclei. He was, however, mistaken in this interpretation, as will be apparent from an examination of my *figs. 5* and *7*. In several instances spherical masses of similar material have been observed in other locations in the cell. These were smaller and their presence did not apparently affect the size of the main masses situated between the centrosome-like bodies and the nucleus, which were also present in the same cell. The reactions of these bodies to stains would indicate that they may be masses of extra-nuclear nuclein.

The results of my studies on *Zamia* and *Ginkgo* are now

<sup>9</sup> On the attraction spheres in the pollen cells of *Ginkgo biloba*. *Ibid.* p. 360; also *Études sur la fécondation et l'embryogénie du Ginkgo biloba*. *Ibid.* p. 318.

<sup>10</sup> *Die Morphologie und Physiologie des pflanzlichen Zellkernes* 107.

sufficiently complete to enable one to judge quite accurately as to the real nature of the centrosome-like body which occurs in the generative cells of these plants. In *Ginkgo* the centrosome-like body cannot be detected in the generative cell until two months after the germination of the pollen grain, when the generative cell has greatly increased in size. It then appears as a very small round body in the cytoplasm between the nuclear membrane and the cell wall. It gradually increases in size, and a month later is found to have grown to three or four times its original diameter. It is thus evident that in *Ginkgo* these bodies originate in the cytoplasm of the generative cell. The early stages of development of the generative cell of *Zamia* have not been studied, and, therefore, in this case their first appearance has not been observed. It has, however, been determined that they increase in size here as in *Ginkgo*. In *Zamia* the centrosome-like body, as described in my previous papers, finally ruptures during the division of the generative cell, and the membrane formed by its wall becomes greatly extended in length, ultimately forming a narrow band arranged in the form of a helioid spiral on one side of the cell. This band gives rise to the cilia which form the motile organs of the antherozoid. In fecundation, as shown above, this ciliiferous band developed from the centrosome-like body is left in the cytoplasm at the apex of the archegonium, while the nucleus wanders alone from that point to the oosphere. The ciliiferous band surely remains intact at the apex of the archegonium for some considerable time after fecundation, and is then gradually absorbed as the embryo develops. In the divisions of the oosphere immediately following fecundation, furthermore, no indication of any body resembling a centrosome could be found. Hirase, who studied the division of the oosphere of *Ginkgo*, was also unable to find any trace of a centrosome. It thus seems quite evident from the above facts that the bodies in question cannot be considered as true centrosomes. The mere fact that during a part of their existence they are situated approximately where a centrosome might be expected to occur if present, and for a considerable time during the resting condi-



tion of the nucleus have the kinoplasmic filaments centered upon them, is not sufficient reason for considering them to be centrosomes, when they differ in many other important features. Their origin, function, and fate are totally different from that of any organ known to the writer which has been considered to be a centrosome. If the bodies in question are compared to typical centrosomes such as occur in *Fucus*, as described by Strasburger,<sup>11</sup> and in *Stypocaulon* as described by Swingle,<sup>12</sup> the dissimilarity of the two organs becomes striking. The two most important features of a centrosome, namely, continuity from cell to cell, and forming the center of an aster at the pole of the spindle during karyokinesis are not shown by the centrosome-like bodies of *Zamia* and *Ginkgo*. In view of our more complete knowledge of the origin, function and fate of the centrosome-like bodies in the two plants under consideration it is evident that they must be considered distinct organs of the protoplasm of spermatic cells, having for their primary function the formation of the motile cilia of the antherozoids. I am not aware that any distinguishing name has been applied to such an organ, and I would here suggest the name *blepharoplast*<sup>13</sup> to distinguish them from other organs of the cell.

In two exceedingly important preliminary papers recently presented before the German Botanical Society, Belajeff has described the occurrence of an organ in the spermatic cells of *Filicineæ*<sup>14</sup> and *Equisetineæ*<sup>15</sup>, which is doubtless identical with the blepharoplasts of *Zamia* and *Ginkgo*. They apparently originate in the spermatic cells, since no trace of them could be discovered in the spermatic mother cell in the resting condition or during karyokinesis. The first changes visible in the metamor-

<sup>11</sup> Kerntheilung und Befruchtung bei *Fucus*. Jahrbücher f. wissenschaftl. Bot. 30: 369. 1897.

<sup>12</sup> Zur Kenntniss der Kern- und Zelltheilung bei den Sphacelariaceen. Jahrbücher f. wissenschaftl. Bot. 30: 326. 1897.

<sup>13</sup> From *βλεφαρις*, eyelash or cilium; and *πλαστος*, formed.

<sup>14</sup> Ueber den Nebenkern in spermatogenen Zellen und die Spermatogenese bei den Farnkräutern. Berichte d. deutsch. bot. Ges. 15: 337. July 1897.

<sup>15</sup> Ueber die Spermatogenese bei den Schachtelhalmen. Berichte d. deutsch. bot. Ges. 15: 339. July 1897.

phosis of the spermatic cells occur in these organs. They gradually become extended into a thread which assumes the form of a helicoid spiral of which the extended turns of the posterior end surround the nucleus. The cilia of the antherozoids are developed from the anterior end of this spiral, appearing first as small protuberances on the thread, which finally become greatly extended and form the cilia. It is remarkable that in the comparatively very small spermatic cells of the Filicineæ and Equisetineæ Belajeff should have been able to trace so accurately the method of formation of the ciliiferous band and cilia. The process which he describes as occurring in these plants is identical in all essential features with that which I have described in *Zamia*, and its publication was almost simultaneous with that of my article.

From previous observations on the spermatogenesis of Characeæ Belajeff is inclined to think that the same organ occurs there also.<sup>16</sup> He further calls attention to the occurrence of somewhat similar organs in the spermatic cells of certain animals.

Belajeff's studies greatly strengthen the view that the blepharoplasts are distinct organs of the cell, differing from centrosomes, and strongly indicates that they occur very generally in the spermatic cells of Filicineæ, Equisetineæ, and Cycadaceæ, if, indeed, they are not of universal occurrence in the spermatic cells of plants and animals.

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#### EXPLANATION OF PLATE X.

##### *Zamia integrifolia.*

FIG. 1. Archegonium immediately before fecundation by the nucleus of the antherozoid, separated from the ciliiferous band and cytoplasm, lying in the protoplasm at the apex: *o*, oosphere; *mn*, male nucleus; *b*, portion of ciliiferous band.  $\times 30$ .

FIG. 2. Antherozoid in cytoplasm at apex of the archegonium, with nucleus separated from the ciliiferous band and cytoplasm.  $\times 100$ .

<sup>16</sup> A more detailed account of Belajeff's discoveries is given under "Notes for Students," p. 302 of this number.

